Annex H

A study of the effects of exposing *Vibrio fischeri* to the proteodies of its Lux A and Lux B genes

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The Lux A and Lux B genes of the microorganism *Vibrio fischeri* were transcribed in the form of two protein melodies ('proteodies'), according to the method described by Joel Sternheimer in his 'Process for the epigenetic regulation of protein biosynthesis by scale resonance', filed under n° 92 06765 at the French *Institut National de la Propriété Industrielle* (INPI). The two proteodies were decoded and connected to each other (Lux A following Lux B) so as to produce the 'Lux AB proteody', which was played in a repeating loop for a given time. The Lux AB proteody was recorded onto a CD-R, using a PC engraver in a Windows 98 environment. The music software used was Cakewalk version 6.0.

Two musical timbres were tried: one low-toned harpsichord-like, which did not work; and one (*a priori* much closer to the correct one, according to the quoted method) which produced sounds like those of tiny bells, giving positive results. As for note lengths, we simply recorded a series of quarter notes with a value of 90 (Cakewalk), amounting to 90 notes per minute.

A positive reaction to this stimulation could be evidenced and assayed by observing an increase in the quantity of light emitted by *Vibrio fischeri*. For practical reasons, we first tried exposing *Vibrio fischeri* to the

proteodies in a liquid medium, but the results were not satisfactory.

Experimental protocol

We developed a method that revealed an increase in light emitted by *Vibrio fischeri* cultivated on agar in an air medium. To do this, we poured agar into glass microtox tubes. The fact that the tubes were made of glass was important. We suspected that tubes made of synthetic materials could modify the results, due to various influences, such as electric-type forces which could be generated (electrostatic, electromagnetic, or other), and possibly disturb the message and/or response of the microorganisms. The tubes were seeded with *Vibrio fischeri* from a mother culture. Measurements were taken after 24 hours. We verified that the thickness of the agar layer did not exceed 3mm, corresponding to 150µl per tube The agar layer had to be sufficiently thin, since the luminometer used in the study read from the bottom up.

Thus, the values presented here were read from below; through a layer of glass (the microtox tubes) and a layer of agar, before reaching the microorganisms themselves.

Experiment N° 1

Two series of tubes were exposed to the proteody. One set was seeded with 15µl of the mother solution and another with 10µl.

For each series:

- The luminosity of 4 tubes was measured at time zero.

- 4 other tubes were exposed for 9 minutes.
- 4 other tubes were exposed for 18 minutes.
- 4 other tubes were exposed for 27 minutes.

The measurements were always carried out immediately after exposure. The luminosity of four unexposed control tubes was also measured each time.

The last 4 tubes were exposed for an additional 9 minutes, and their luminosity measured again.

Exposure was carried out as follows: The tubes were opened at the last moment and inclined at a 45° angle. The proteodies were played on a portable laser-disc player (Clip Sonic, model n° 1119) whose speakers were aimed directly at the open tubes at a distance of 90cm. The disc player was not positioned nearer to the tubes because of magnetic fields it emitted, especially by its loudspeakers. The sound volume (gain) was adjusted to one-third maximum. The microtox tubes and the laser disc player were raised 3cm above the table surface, so as to avoid being too low with respect to the loudspeakers.

Results of experiment n° 1

***Vibrio fischeri* exposed to LuxAB proteody**

control 1
music 1
Luminometer values
Exposure time
Luminometer readings
Series
Control

Mean

Interpretation

It was possible to observe that the proteodies triggered a luminous reaction in direct proportion to the time of exposure. The relation 'stimulation by proteody/luminous activity' was very strongly significant. Nevertheless, at 27 + 9 minutes, a decrease in luminosity was observed for the 15 µl solution, which must be studied by means of additional trials. *A priori* this was either an artefact or a saturation-type reaction due to the higher number of microorganisms in the second series of tubes.

Experiment N°2

Various tubes were exposed to the Lux AB proteody for 15 seconds or 9 minutes. A measurement was taken first at t=0, then at various other times, in order to observe the evolution in luminosity.

Results of experiment n°2

***Vibrio fischeri* exposed to LuxAB proteody for 15 seconds at t=0 {9 minutes}**

Evolution of luminosity

15 seconds of stimulation (music 1)
9 minutes

Interpretation

It was clear that we observed:

- A general correlation between time elapsing and progressive reduction in *V. fischeri* luminous activity;
- A correlation between the duration of stimulation (i.e. of exposure to LuxAB proteody) and the intensity of *V. fischeri* luminous activity.

Once again, the relation 'stimulation by proteody / luminous activity' was very strongly significant.

Conclusion

In the work described here, the luminous activity of *Vibrio fischeri* was stimulated by stimulation of the Lux A and Lux B genes of that same organism, via a series of audible frequencies (LuxAB proteody).

The results obtained show that it is possible to stimulate a protein synthesis in a highly significant way by using proteodies.

Numerous parameters have not been perfectly mastered, such as (among others):

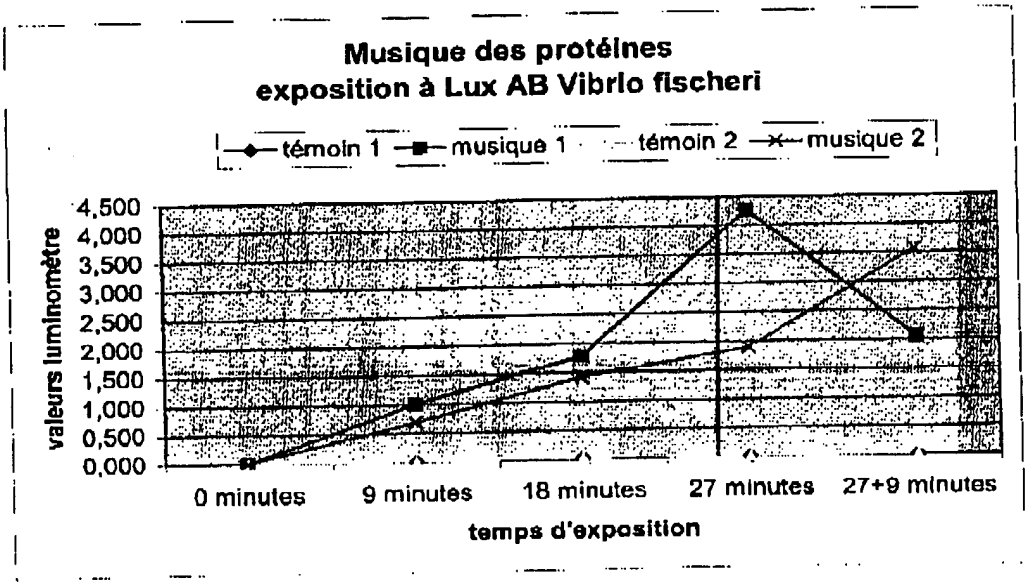
- melody
- timbre
- rhythm
- the exact frequency assigned to each amino-acid
- sound volume
- frequency preferences of the organism studied (low

frequencies, ultrasound, light)
- appropriateness in the selection of selected genes
- interactions between/among the various genes
- etc...

Considering the fact that the results obtained were very highly significant under non-optimal experimental conditions, it is possible to deduce that living organisms seem to react in an extremely rapid and strong manner to gene stimulation delivered via proteodies.

It would appear that we have observed a key mechanism involved in the functioning of living organisms.

Résultats expérimentaux de l'expérience n°1



Résultats expérimentaux de l'expérience n°2